

## BEHAVIORAL TERATOGENICITY OF METHAMPHETAMINE

Kang Chin, Dae Hyun Cho and Tae Soon Cho\*

*National Institute of Safety Research, Seoul 122-020, Korea*

*\*Sung Kyun Kwan University*

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**ABSTRACT:** *Pregnant Wister rats were given daily subcutaneous administrations of methamphetamine (MAPT; varying doses ranging from 1.0 to 4.5 mg/kg) from days 7 to 20 of gestation and teratogenic effects have been determined. The teratogenic effects inducible with orally administered caffeine (90 mg/kg/day) for the same durations were used as the positive controls. MAPT doses greater than 2.0 mg/kg have suppressed the rate of maternal weight gain. Some of the offsprings (F1) of the prenatal MAPT treated groups had decreased growth rate and delayed development of physical characters and functional reflexes. The male offsprings of the MAPT treated groups had significant decreases in their spontaneous motor activity but had enhanced conditioned avoidance responses. However, the mating performances of these offsprings were not affected. These results indicated that prenatal exposure of MAPT may induce some behavioral teratogenicity in rats.*

**Key words:** *Methamphetamine, prenatal exposure, rat, behavioral teratogenicity.*

### INTRODUCTION

Amphetamine and methamphetamine produce strong psychostimulant effects both in humans and experimental animals. In humans, these psychostimulant drugs have been widely abused because they produce the feeling of well-being and euphoria. These drugs appear to produce the psychostimulant effects by enhancing the release of monoamines from the nerve terminals and also by inhibiting the reuptake of the released neurotransmitter back to the nerve terminals in the brain. In experimental animals, the amphetamines cause increased alertness, locomotor activity as well as the aggressive behavior. Repeated treatment of newborn animals with methamphetamine has been shown eventually to inhibit the catecholamine biosynthesis and depletes the dopamine and norepinephrine from brain, abolishes the psychostimulant effect of methamphetamine given at a later time. These results suggested that repeated self administrations of methamphetamine by pregnant mothers may bring about altered levels of monoamine neurotransmitters in the fetal brain and produce behavioral

teratogenicity. However, teratological effects of methamphetamine have not been well characterized. Results of few existing behavioral teratogenicity studies on methamphetamine are not comprehensive and, at best, controversial (Martin *et al.*, 1975; 1976; 1979; 1981; Watanabe *et al.*, 1985; Sato and Fujiwara, 1986). For example, Watanabe *et al.* (1985) reported that MAPT do not produce any behavioral teratogenicity, but Martin's group (1981) have reported an increased locomotor activity and Sato *et al.* (1986) have reported a decreased motor activity in offsprings born from pregnant rats exposed to MAPT.

Thus, in the present study, we have attempted to re-examine the behavioral teratogenicity of methamphetamine in a more comprehensive manner by determining the growth rate, developments of physical characteristics and neuromuscular reflexes, spontaneous motor activity and conditioned avoidance responses of the offsprings. At last, we have even examined the sexual and reproductive performances of the offsprings.

## MATERIALS AND METHODS

Five week old male and female SPF Wistar rats were obtained from the vivarium of Korean NIH. The animals were acclimatized to the laboratory conditions by being housed in an environmentally controlled room (temperature:  $23 \pm 2^\circ\text{C}$ ; humidity:  $55 \pm 5\%$ ; 12 hr light from 7:00 to 19:00) and placed in polycarbonate cages (1 per cage) for 7 weeks. They were allowed free access to a laboratory chow and tap water.

Two female rats were mated with a male rat in the same cage over-night. The day on which the vaginal plug was observed or sperms were detected in the vaginal smear was regarded as day 0 of gestation. Dams were arranged in groups and were given subcutaneous administrations with methamphetamine HCl (NIH, Korea dissolved in saline at 1.0, 2.0, 3.0 or 4.5 mg/kg/day (dosage volume: 5 mg/kg) for fourteen days from days 7 to 20 of gestation. Each group consisted of 13 to 14 dams. For the positive and negative control groups of testing the behavioral teratogenicity, caffeine (Sigma Chemical Co; 90 mg/kg/day; dosage volume: 10 mg/kg) and saline, respectively, were also given to dams by oral and subcutaneous administrations for the same durations.

Dams in each group was examined daily for general signs of health, mortalities, and body weight gains. Dams were allowed to deliver and nurse their young until 4 days after delivery. At parturition (regarded as postnatal day 0), the number of newborn per dam was counted, sexed, weighed and examined for external anomalies. On day 4, 8 pups (4 male and 4 female) were selected at random from each litters and the development of physical characters as well as the functional (physiological) reflexes were examined throughout the lactation period according to the published methods of Irwin (1968), Altman and Sudarshan (1975), Brunner *et al.* (1978) and Butcher *et al.* (1980). Beginning from day 3, selected pups were observed daily for the completion for pinna detachment, appearance of abdominal hairs, lower incisor eruption, eye opening, and testes descent or vagina opening.

The behavioral (reflex) development and conditioned avoidance response of pups were examined according to the published methods of Boulton *et al.*, (1982), Dourish

*et al.* (1983), and Omori *et al.* (1985). Beginning from day 4, presence of surface righting reflex has been tested by the 2 sec return to prone position of the turned over pup. Also, cliff avoidance test has been performed by placing the pup on an edge with forepaws and nose over the edge and waited for the showing of sideward movements and retraction backward between 10 to 30 sec for completion. In addition, the presence of 25' negative geotaxis reflex was tested twice a day from day 6 to 12 by measuring the time required to complete the process of moving from head-down to head-up rotation within 60 sec. Furthermore, beginning on day 12, pups were held in an inverted position 56 cm above a flat cushion surface and quickly released to test for the presence of mid-air righting reflexes. The pup's ability to right itself prior to reaching the surface was monitored visually.

To examine the spontaneous motor activity, pups were placed individually in a Opto-Varimex activity monitoring cage (Columbus Instrument, USA) beginning from 3 weeks after birth. The measurement items consisted of distances traveled, ambulatory times, stereotypic movement times, resting durations and the numbers of small movements.

Conditioned avoidance response (CAR): Beginning at 7 weeks after birth, the offsprings were placed in a shuttle box designed for measuring the conditioned avoidance responses (CAR; Muromachi Kikai Co., Japan). The durations required for acquisition of CAR and the latency time for the CAR have been determined. The shuttle box consisted of two compartments and it was set for 20 sec inter-trial interval (ITI) followed by a 5 sec light-on period before delivering the 2.5 mA and 3 sec duration electric shock to the base grid (unconditioned stimulus; US). The time required to avoid the electric shock was noted and a total of 30 trials were performed with each animal. An avoidance response would reset the ITI clock. The dependent measures have included: 1) avoidance during the preshock period, 2) avoidance during the conditioned stimulus-unconditioned stimulus (CS-UCS) interval, and 3) escape response.

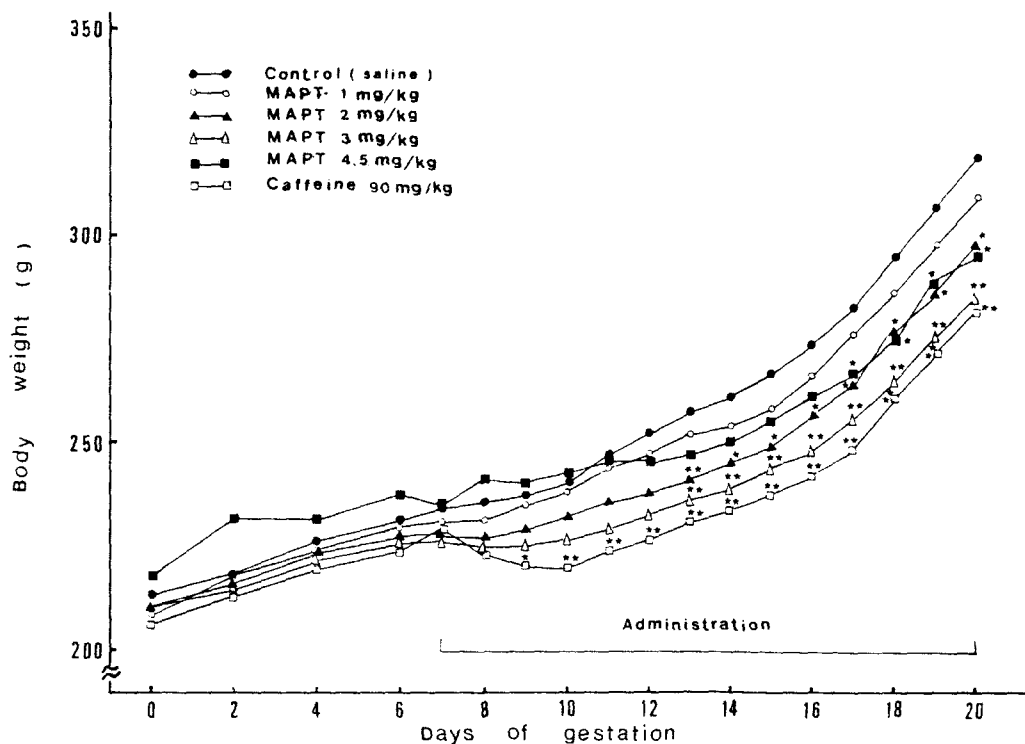
The reproductive performances of the male and female offsprings were assessed after 14th week of postpartum. The male and female offsprings were mated individually for 2 weeks.

## RESULTS

The rates of body weight gain for each of the MAPT treated groups during gestation are shown in Fig. 1. The rates of maternal body weight gain were significantly suppressed after the sixth daily administrations of MAPT given at 2.0 and 3.0 mg/kg and after the tenth administrations of MAPT at 4.5 mg/kg. In the group treated with 4.5 mg/kg MAPT, only 1 out of 13 dams delivered live newborn. However, for other groups treated with lower doses of MAPT, the live birth index (%) and the mortality rates were not significantly different from those of the controls.

During lactation and post weaning periods, the rates of body weight gain for the male offsprings were significantly suppressed for the group treated with MAPT at 3.0 and 4.5 mg/kg (Fig. 2).

When the durations for appearance of various physical characters such as lower incisor eruption, eye openings, and testes descent were determined, they were



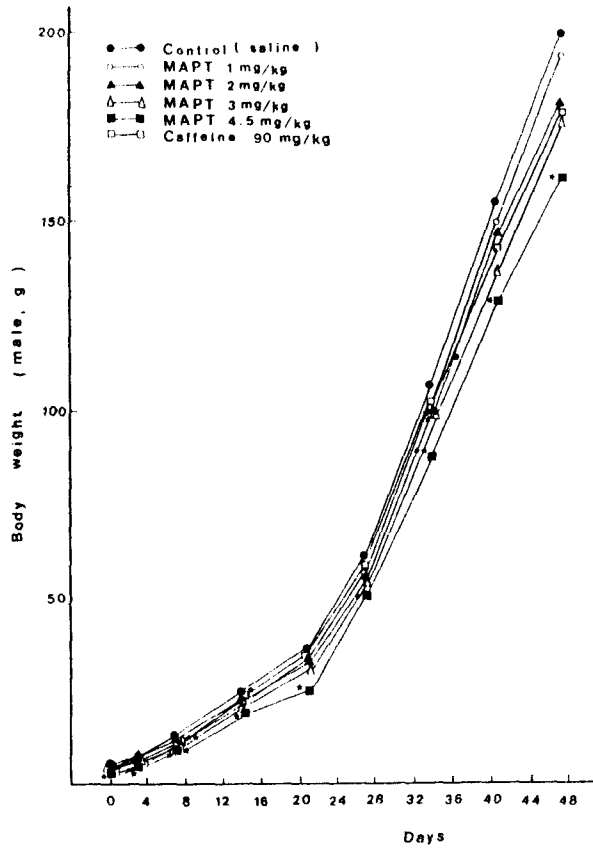
**Fig. 1.** Mean body weight changes (g) of dams during gestation <sup>a</sup>;  $P < 0.05$ , <sup>\*\*</sup>;  $P < 0.01$ . Significant difference from control.

**Table 1.** Physical development of rat offspring ( $F_1$ )

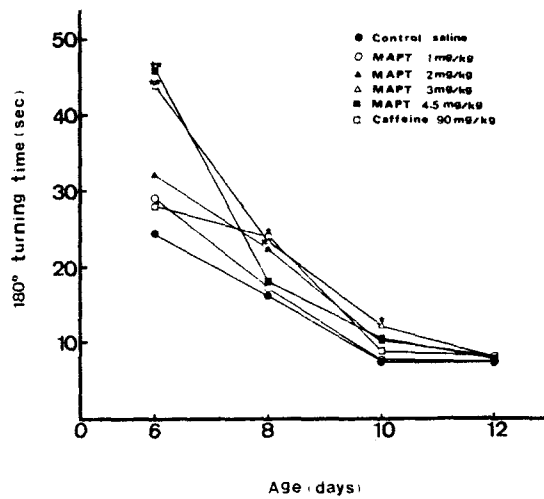
Compound	Control		MAPT			Caffeine
	Saline	1	2	3	4.5	90
Dose (mg/kg)	Saline	1	2	3	4.5	90
No. of litters	12	10	11	9	1	11
Detachment of pinna	$3.1 \pm 0.5^{a)}$	$3.2 \pm 0.6$	$3.1 \pm 0.9$	$3.3 \pm 0.6$	$3.2 \pm 0.5$	$3.4 \pm 0.7^*$
Appearance of abdominal hairs	$9.3 \pm 0.7$	$9.2 \pm 0.4$	$9.3 \pm 0.8$	$9.5 \pm 0.5$	$9.4 \pm 0.4$	$9.5 \pm 0.8$
Eruption of incisor	$10.4 \pm 0.7$	$10.5 \pm 0.5$	$10.5 \pm 0.5$	$10.7 \pm 0.7^*$	$10.6 \pm 0.4$	$10.7 \pm 0.5^*$
Opening of eyelids	$14.4 \pm 0.5$	$14.6 \pm 0.6$	$14.6 \pm 0.5$	$14.7 \pm 0.7^{**}$	$14.6 \pm 0.7^*$	$14.8 \pm 0.5^{**}$
Descent of testis	$22.7 \pm 0.9$	$22.6 \pm 0.5$	$23.2 \pm 0.8$	$23.9 \pm 0.7^*$	$23.4 \pm 0.5$	$23.2 \pm 0.6$
Opening of vagina	$34.1 \pm 1.2$	$35.2 \pm 1.8$	$35.0 \pm 1.8$	$35.0 \pm 1.4$	$35.8 \pm 1.0$	$36.8 \pm 1.7^*$

<sup>a)</sup>; Mean  $\pm$  S.D. (Days)

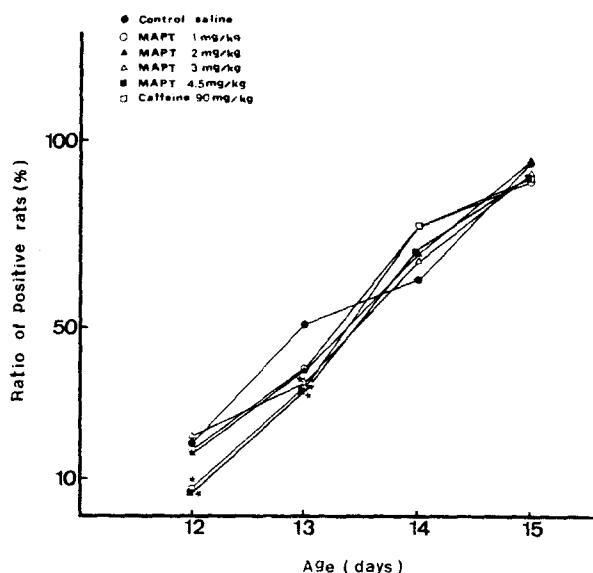
\*;  $p < 0.05$ , \*\*;  $p < 0.01$ , Significant difference from control.



**Fig. 2.** Mean body weight changes of F<sub>1</sub> male (g). \*; P<0.05. \*\*; P<0.01. Significant difference from control.



**Fig. 3.** Negative geotaxis (NG) of rat offspring (F<sub>1</sub>). \*; P<0.05. \*\*; P<0.01. Significant difference from control.



**Fig. 4.** Mid-air righting reflex (MRR) of rat offspring ( $F_1$ ). °;  $P < 0.05$ . °°;  $P < 0.01$ . Significant difference from control.

**Table 2.** Spontaneous motor activity in offsprings ( $F_1$ ) at 3 weeks

Compound (mg. kg)	Dose	No. of offspring ( $F_1$ )	Spontaneous motor activity (counts/ 6 min.)					B.W. <sup>6)</sup>
			D.T. (cm) <sup>1)</sup>	R.T. (sec.) <sup>2)</sup>	S.T. (sec.) <sup>3)</sup>	A.T. (sec.) <sup>4)</sup>	S.M. <sup>5)</sup>	
Control (Saline)	—	16	1754.4 ± 302.7 <sup>a)</sup>	94.0 ± 20.2	167.2 ± 11.0	98.5 ± 12.8	417.0 ± 32.7	59.9 ± 7.8
MAPT	1	13	1717.6 ± 288.5	89.1 ± 13.9	172.0 ± 8.0	98.8 ± 13.1	426.5 ± 23.9	55.8 ± 7.8
MAPT	2	14	1435.6 ± 313.5**	110.9 ± 31.4	165.0 ± 17.0	84.3 ± 16.9*	397.8 ± 47.8	62.0 ± 10.8
MAPT	3	8	1477.4 ± 345.0*	101.2 ± 15.9	165.8 ± 11.3	92.8 ± 9.9	385.9 ± 126.1	57.9 ± 8.8
MAPT	4.5	8	1358.0 ± 55.1**	116.3 ± 3.5*	165.0 ± 7.0	78.7 ± 3.2*	393.7 ± 3.1	42.7 ± 9.1**
Caffeine	90	22	1452.1 ± 337.1**	105.2 ± 27.0	170.0 ± 16.3	84.8 ± 16.9*	405.3 ± 46.2	59.4 ± 7.4

<sup>a)</sup> Mean ± S.D.

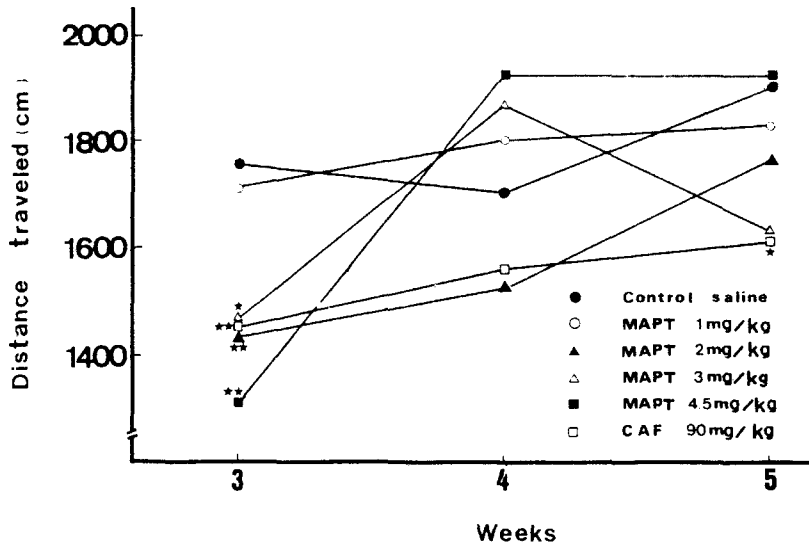
1. D.T.; Distance Traveled, 2. R.T.; Resting Time, 3. S.T.; Stereotypic Time, 4. A.T.; Ambulatory Time, 5. S.M.; No. of Small Movement, 6. B.W.; Body Weight.

\* Significantly different from control group ( $p < 0.05$ )

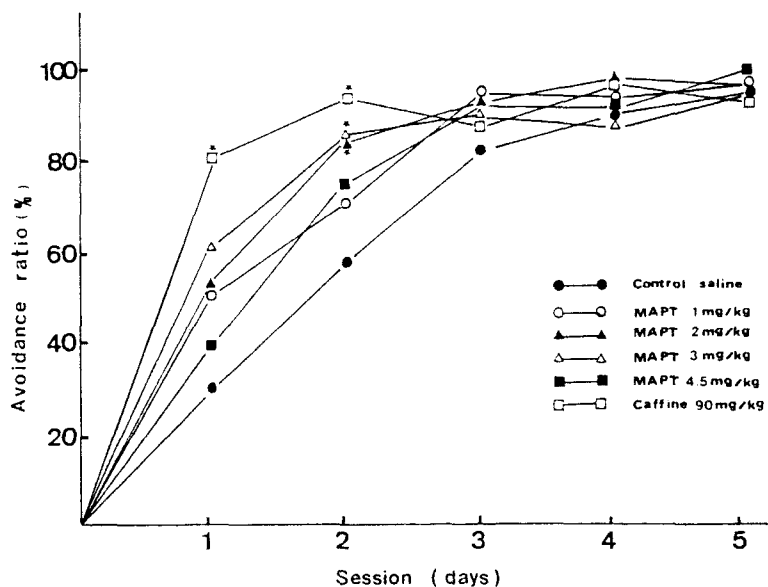
\*\* Significantly different from control group ( $p < 0.01$ )

significantly delayed in the group treated with 3.0 mg/kg (Table 1).

The functional developments, as measured by the presence of surface righting reflex and cliff avoidance responses, were not affected at all by the prenatal exposure to MAPT and were completely developed between 4 and 5 days after birth in all offsprings. There were no significant differences between the control and treatment groups.



**Fig. 5.** The changes of spontaneous motor activity in offspring ( $F_1$ ). \*:  $P < 0.05$ , \*\*:  $P < 0.01$ . Significant difference from control.



**Fig. 6.** The acquisition of avoidance response in offspring ( $F_1$ ) at 7 weeks. \*:  $P < 0.05$ . Significant difference from control.

However, the durations required for the development of negative geotaxis reflex was significantly delayed in the 3.0 and 4.5 mg MAPT/kg treated groups. While the delays were noted until days 6, 8 and 10 of birth, beginning from days 12, no significant differences were noted for all of the offsprings in MAPT treated groups (Fig. 3). Also, the results of mid-air righting reflex shown in Fig. 4 indicate that there are significant delays

**Table 3.** Effect of MAPT on latency time of avoidance response in shuttle box test

Compound	Dose (mg/kg)	No. of offspring (F <sub>1</sub> )	Latency time (sec.)				
			1st	2nd	3rd	4th	5th day
Control	—	6	2.11 ± 0.37 <sup>a)</sup>	2.44 ± 0.83	1.96 ± 0.39	2.26 ± 0.19	2.10 ± 0.36
MAPT	1	7	2.36 ± 0.32	2.19 ± 0.47	1.91 ± 0.38	2.18 ± 0.22	2.28 ± 0.50
MAPT	2	7	2.40 ± 0.50	2.09 ± 0.27	2.07 ± 0.33	1.95 ± 0.34*	2.02 ± 0.29
MAPT	3	6	2.37 ± 0.33	1.99 ± 0.30	2.10 ± 0.35	2.27 ± 0.32	2.00 ± 0.23
MAPT	4.5	6	1.80 ± 0.93	1.84 ± 0.35*	1.96 ± 0.39	1.95 ± 0.18*	2.05 ± 0.31
Caffeine	90	7	2.19 ± 0.45	1.79 ± 0.25*	2.09 ± 0.19	2.04 ± 0.34	1.88 ± 0.44

<sup>a)</sup> Mean ± S.D.

\*P < 0.05. Significant difference from control.

for its development in the offsprings of 3.0 and 4.5 mg/kg MAPT groups on day 12 and 13.

Results of spontaneous motor activity of offsprings at 3 weeks after birth are shown in Table 2. While the total motor activity indices as determined by DT, AT and SM were reduced, the resting durations for the MAPT-pups were greater than that of the pups in the control group. The chronological developmental patterns of total spontaneous motor activity as indicated by the distances traveled for pups of 3 to 5 week old are shown in Fig. 5. The total distances traveled for pups of 2.0, 3.0 and 4.5 mg/kg MAPT treated groups were significantly less until week 3 of post partum. However, the results obtained with 4 and 5 week old pups did not show any statistically significant differences. The decreased total motor activity and increased resting durations observed in 3 week old pups were reasonably consistent and these are believed to have resulted from prenatal exposure to methamphetamine.

The avoidance response rates and latency time for development of avoidance responses are shown in Fig. 6 and Table 3, respectively. All offsprings of the MAPT treated groups tended to have higher avoidance response rates than those of the control group from the 1st through 3rd session (days). The increases in the avoidance response rates and the decreased latency time observed for the offsprings are believed to have resulted from the prenatal exposure to methamphetamine.

The mating performances of male and female offsprings were not affected by the prenatal exposure to MAPT.

## DISCUSSION

Results of the present experiment have provided evidences that prenatal MAPT exposures have induced behavioral impairments and growth retardations in the offsprings. Prenatal exposures to MAPT have induced reductions in the rate of body weight gain, delays of physical developments (incisor eruption, eye opening, testes descent) and delays of functional (reflex) developments (NG, MRR). These results appear to have confirmed the findings of Martin *et al.* (1975, 1976) who have reported retardations of growth rate and delays of eye opening upon gestational and nursing exposures of 5 mg/kg/day MAPT in rats.



The prenatal exposures to MAPT appears to affect the development of reflex behaviors particularly for those offsprings in early days after birth. Prenatal exposure to MAPT have also affected the development of spontaneous motor activity by reducing the distances traveled, ambulatory times and the numbers of small movements as well as increasing the resting durations. While Martin and Martin (1981) reported similar results, Sato *et al.* (1986) have reported decreases of the total motor activity with increased vertical activity, and yet, Watanabe *et al.* (1985) have reported that the development of these reflex behaviors in the offspring were not affected at all by the repeated prenatal exposures to MAPT.

Prenatal exposures to MAPT have produced more significant effects on the development of conditioned avoidance responses particularly in the 3.0 mg/kg MAPT treated group. Our results have confirmed the findings of Martin *et al.* (1975) who have reported a significant increase of conditioned avoidance responses for the offsprings of 5 mg/kg MAPT treated rats.

In view of these results, authors believe that this comprehensive teratogenicity study with MAPT is one of the few studies which have demonstrated a significant behavioral changes following prenatal exposure to MAPT reporting on the growth, physical, and reflex development, spontaneous motor activity, conditioned avoidance response, and reproductive performance of offsprings. In conclusion, methamphetamine might induce some behavioral teratogenicity in rats.

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